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## ABSTRACT

### EVALUATING A MEASLES AND RUBELLA MULTIPLEX BEAD ASSAY FOR COUNTRIES IN THE WHO GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK

By

ALEXANDRIA R. MITCHELL

**INTRODUCTION:** Measles and rubella are highly contagious viral diseases. Measles remains one of the leading causes of vaccine-preventable deaths, and rubella during pregnancy can cause congenital rubella syndrome. The enzyme immunoassay (EIA) is most frequently used to determine antibody responses to measles and rubella, and the plaque reduction neutralization assay (PRN) is considered the gold standard for determining measles immunity. A measles and rubella multiplex bead assay (MBA) is currently being evaluated at the Centers for Disease Control and Prevention for its utility in serosurveillance within the World Health Organization Global Measles and Rubella Laboratory Network, and the MBA has several benefits over these assays. The MBA requires a smaller sample volume, measures several diseases simultaneously, is faster, and has less technician-dependency than the PRN.

**AIM:** Study objectives are to compare accuracy of measles serological status from both the MBA and EIA when compared with the PRN, and to evaluate whether country or participant age are associated with test accuracy.

**METHODS:** Samples for participants of varying ages from the United States, Tajikistan, and Bangladesh (n=300) that had been tested by each assay were used. Results were dichotomized as positive or negative according to respective assay cutoff values. Logistic regression models were applied to estimate point estimates and confidence intervals for sensitivity and specificity of the MBA and EIA with relation to the PRN gold standard.

**RESULTS:** Across the three age groups and countries, EIA has higher median values (mIU/ml) compared with MBA and PRN (582.55, 399.28, and 378.95, respectively, for Tajikistan participants aged 13 and older). McNemar's Test of Agreement comparing MBA and PRN suggests disagreement ( $p < 0.0001$ ). This is also shown with EIA and PRN ( $p < 0.0001$ ), but not when MBA and EIA were compared ( $p = 0.4669$ ). MBA and EIA have similar overall sensitivities, with 97.29% (94.09, 98.76) and 97.76% (94.73, 99.06) when each compared to the gold standard PRN. MBA and EIA sensitivities were high, and specificity improved from 42.86% and 40.26%, to 73.02% and 65.56%, respectively, when source country was added. They further improved to 77.55% and 73.69%, respectively, when age was added to the model. For the United States, sensitivity for the MBA is higher for 0-5 and 13+ year age groups at 87.22% and 95.64%, respectively, compared with 84.37% ( $p = 0.8650$ ) and 94.29% ( $p = 0.8103$ ) for EIA.

**DISCUSSION:** When compared with the PRN, the MBA has similar accuracy to the EIA with regards to sensitivity and specificity, and in several groupings of country and age, these percentages are higher for the MBA. Many of the benefits of the MBA over conventional assays have led to its increased application in research, and these results suggest the MBA can be used in settings where the EIA is currently used.

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GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK

by

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B.A., EMORY UNIVERSITY

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of Georgia State University in Partial Fulfillment  
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APPROVAL PAGE

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#### Author's Statement Page

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 June 26, 2018

Alexandria R. Mitchell

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# 1 INTRODUCTION

## 1.1 Background

Measles and rubella are highly contagious viral diseases most often associated with fever, runny nose, cough, conjunctivitis (red eyes), and a rash. In 2009, the WHO recommended a two-dose measles, mumps, and rubella (MMR) vaccine for children (Rota et al., 2016), but measles remains one of the leading causes of vaccine-preventable deaths. An estimated 100,000 or more children die annually from measles (Rota et al., 2016), with an estimated 89,780 measles deaths in 2016 (Dabbagh, 2017). Rubella also remains a global threat, and can cause congenital rubella syndrome (CRS) during pregnancy, with an estimated 300 children globally who are born daily with lifelong cardiac and ocular diseases, or hearing impairments (Dabbagh, 2017). Despite vaccination campaigns and efforts, outbreaks and vaccine failures can still occur; serosurveillance methods are used to monitor and confirm a country's disease elimination status (Durrheim, Orenstein, & Schluter, 2018; Mulders et al., 2016).

Currently, the enzyme immunoassay (EIA) is most frequently used to determine antibody responses to measles and rubella. However, the EIA is a single-antigen platform limited to only measuring one disease at a time. As such, it can be time consuming and costly; the EIA also uses a large volume (10ul, ZEUS Scientific, Inc.) of sample. The plaque reduction neutralization assay (PRN) is the gold standard (reference) in measuring seroprotection, but is technician-dependent and time consuming, and requires more volume (60ul) than the EIA. Seroprotection refers to the antibody response sufficient to protect a person from getting the disease; samples that are not seroprotected could be seropositive or seronegative, and those subjects are potentially susceptible to disease. Regarding the PRN, seroprotection corresponds

to neutralizing antibody concentrations above 120mIU/ml (Chen et al., 1990). Due to the training and time required for the PRN, there are few countries with the resources and personnel available to use the PRN for analyzing samples, making this platform impractical for serosurveillance studies. Concerning susceptibility of disease in these studies, it is meaningful to determine measles seroprotection for separate age categories. If any age groups have lowered immunity this will be apparent with separation of ages. Children under 6 years are within the CDC recommended period to receive the two-dose MMR vaccine (CDC, 2018); the first dose is recommended for ages 12 to 15 months, and the second dose between four and six years of age. Adults aged 21 and older are more likely to have already developed antibodies against measles either from natural disease or from receiving the vaccine. Countries may have different vaccination recommendations and dose requirements, which are based on the likelihood of contracting the disease. In an urban area of low measles vaccine coverage, infection usually occurs in infants and young children. As coverage increases, the average age of infection shifts towards adolescents and adults who may not have received the vaccine and were not infected as children (Moss, 2017).

At the United States Centers for Disease Control and Prevention (CDC), a Measles and Rubella Multiplex Bead Assay (MBA), sometimes referred to as a multiplex immunoassay (MIA), is currently being evaluated for its utility in generating serosurveillance data. The assay was originally developed at National Institute for Public Health and the Environment (RIVM) in the Netherlands for country serosurveillance (Smits, van Gageldonk, Schouls, van der Klis, & Berbers, 2012), and was then transferred to CDC for evaluation and optimization. The measles team in the Viral Vaccine Preventable Disease Branch (VVPDB) at CDC is a Regional Reference

Laboratory and Global Specialized Laboratory, and aims to assess the MBA for implementation in countries within the World Health Organization (WHO) Global Measles and Rubella Laboratory Network. The MBA is a Luminex flow cytometry-based platform that allows for multiplexing of antigens; this enables researchers to test for the presence of antibodies against several diseases simultaneously, which is cost-effective (Elshal & McCoy, 2006) for serosurveillance studies. The MBA is a direct-antigen assay using microsphere beads covalently bonded to either measles or rubella whole virus antigen. If IgG antibodies to measles or rubella are present in human serum samples, they will “attach” to the antigen on the beads. A fluorescently-tagged secondary antibody added will adhere to IgG antibodies present in the serum sample, and based on a standard curve, the fluorescence unit is converted into the observed concentration of antibodies present in the sample. When a serum sample is assessed with the Luminex machine, the output is given as the observed concentration of antibodies to measles and rubella, respectively. Compared to the PRN and EIA, the MBA requires a much smaller volume of sample (1ul), and is less technician dependent, regarding interpretation of results, than the PRN. Because of its cost-effectiveness, time and sample-efficiency, and multiplex design, the MBA could be implemented as an alternative platform for the EIA or PRN. Previous studies of other diseases have shown the MBA compares well with the EIA (Binnicker, Jespersen, & Rollins, 2011; Gwyn et al., 2017; Reder, Riffelmann, Becker, & Wirsing von Konig, 2008; Smits et al., 2012), but this study seeks to compare the MBA from CDC to both the EIA and the PRN, and additionally assess whether a sample’s country of origin or age influences the MBA’s accuracy.

## **1.2 Statistical Framework**

Differences across source countries can be difficult to describe or quantify, and include varying natural disease prevalence rates, vaccination campaigns, coverage, and failure, disease elimination status, sample collection and storage, access to healthcare, population immunity, vaccination recommendations, and inaccurate or incomplete data (Durrheim et al., 2018). These differences could hypothetically influence the MBA's performance (Fujii et al., 2014). Three serum panels consisting of samples run on the MBA, PRN, and EIA are evaluated in this study, and these panels have varying countries of origin and age information, which are included in the analysis. A logistic regression framework is the most appropriate statistical methodology to use (Agresti, 2007), as this allows for examining the predictive value of country and age, and simultaneous comparison of the MBA and EIA to the gold standard PRN.

## **1.3 Study Aims**

Objectives for this study are to compare the MBA and EIA each with the gold standard PRN platform. The concentration of measles antibodies in human serum samples run on each assay will be used for this comparison. Logistic regression models will be fit to understand how seroprotection by MBA and EIA relates to that of PRN. Sample country and age will be included in the models to assess the predictive value of each on assay comparison.

## **2 REVIEW OF LITERATURE**

Several statistical methodologies have been applied to examine measles immunity, although no other statistical analysis of measles seroprotection, determined by MBA, PRN, and

EIA was found at the time of this study. Only a study using a Markov chain Monte Carlo (MCMC) approach to estimate the effect of vaccination campaigns on the disease burden of measles (Trentini, Poletti, Merler, & Melegaro, 2017) was found in terms of similarity. The MCMC study utilized an innovative statistical methodology using outcomes of previous measles vaccine studies to estimate future study outcomes. However, this assay comparison study will serve as a pilot or feasibility study, guided by previous studies comparing the MBA and EIA, and EIA and PRN found in the literature.

After development of the MBA at RIVM, the assay was compared to the in-house Enzyme-Linked Immunosorbent Assay (ELISA), which is a form of EIA. The MBA and ELISA show a strong positive correlation ( $R^2 = 0.98$ ) with a correlation curve to plot antibody concentration on a log-scale [(Smits et al., 2012) and unpublished data by Coughlin, M, 2016]. Another study (Dorigo-Zetsma et al., 2015) used four EIA kits to determine vaccine-induced measles immunity and evaluated these results against PRN and a measles MIA. Results showed “limitations in the usefulness of current EIA assays for determining protective measles antibodies in persons with a vaccination history.” When compared to the PRN, the MBA also showed a strong correlation (unpublished data by Coughlin, M, 2016). A measles, mumps, rubella, and varicella (MMRV) MIA, has also shown good similarity when compared to the EIA platform, with 93% overall agreement in a shorter time span of 1.7 hours compared to 5.5 hours by EIA (Binnicker et al., 2011)].

MBA and ELISA comparative studies have also been conducted for other vaccine antigens. A newly-developed MBA for detection of tetanus, diphtheria, and pertussis toxins was compared to three traditionally used ELISAs, and suggested the MBA was highly correlated

with the ELISAs (Reder et al., 2008). For tetanus and pertussis toxins the MBA had a positive association with the ELISA, with regression coefficients of 0.910 and 0.905, respectively. For diphtheria toxin, the MBA and ELISA had a regression coefficient of 0.938; these examples all considered samples originating from a single country (pg. 746-747). While this can be advantageous, the measles and rubella MBA will be used in a range of countries and to test whether performance is consistent across these, data from multiple countries should be evaluated. Additionally, using a single country could hypothetically limit information provided about the assay and samples, because it assumes underlying characteristics are the same for all countries.

Other MBA and ELISA comparisons in the literature utilize sensitivity and specificity of each assay to assess overall agreement. For the neglected tropical disease trachoma caused by the *Chlamydia trachomatis* bacterium, the MBA has a sensitivity of 93.2% and specificity of 97.4% (Gwyn et al., 2017). Sensitivity and specificity for the ELISA freshly-coated with antigen is 93.2% and 98.1%, and these were estimated using receiver operator characteristic (ROC) curve analyses on positive and negative reference samples. Using cutoff values generated from the ROC curve, the MBA and ELISA had 86.1% agreement for positive samples and 92.8% agreement for negative samples. Additionally, the two platforms were compared with a lateral flow assay (LFA), where antibodies to trachoma bind to a conjugate pad rather than a bead or plate. Because there is no gold standard for serologic testing to determine disease status for trachoma, the MBA and ELISA platforms were compared further using latent class analysis (Wiegand et al., 2018). This modeling approach uses disease status given by each assay to conclude the probability of a subject belonging to the positive, indeterminate, and negative

disease latent classes. The model with these three classes had the best fit for the data and had similar estimates for sensitivity and specificity of the MBA and ELISA. Sensitivity and specificity of the MBA estimated in the latent class model are both 98%, and for the freshly-coated ELISA 90% and 97%, respectively. These values are higher, and have wider confidence intervals, when the data are fit using a modeling approach compared with the prior application of a ROC curve. This is most likely because there is no gold standard for serologic testing for the bacterium *Chlamydia trachomatis* to determine trachoma disease status, and the latent class modeling allows for consideration of the uncertainty. The studies for trachoma considered samples with three different countries of origin and three assay platforms, which is similar to the study described in this project. However, in this assay comparison study the PRN is a serologic gold standard for determining measles antibody concentration, and separate logistic regression models can be fit to compare the accuracy of the MBA and EIA platforms with PRN. Additional models can be fit to evaluate country and age their respective influence on this accuracy.

A study concerning vaccination coverage of children in India utilized a multinomial logistic model to compare vaccination status, and better understand the predictive value of socioeconomic status and effects like sociodemographic characteristics (Shrivastwa, Gillespie, Kolenic, Lepkowski, & Boulton, 2015). Comparisons were based on Universal Immunization Program (UIP) recommended single dose of bacillus Calmette–Guérin, single dose of measles containing vaccine, and three doses of diphtheria–pertussis–tetanus, with fully-, under-, and non-vaccinated categories. Under-vaccination and non-vaccination categories were compared to the fully-vaccination category, and the authors found religion and caste fixed effects were significant predictors of vaccination status. Compared to Hindu children, the odds of being non-

vaccinated compared to being fully-vaccinated is 2.2 times higher for Muslim children and the odds of being under-vaccinated compared with fully vaccinated is 1.42 times higher.

Several studies have also compared measles IgG antibody levels by the EIA to the gold standard PRN, using several EIA kits. The Measalisa kit for EIA was compared with PRN, and had 99.0% sensitivity and 100% specificity (Neumann, Weber, Jessamine, & O'Shaughnessy, 1985). Similarly, the EIA has demonstrated high sensitivity and specificity (100% and 90.7% for the Enzygnost [Siemens] kit (Ratnam et al., 1995)) when using 120mIU/ml as a cutoff for seroprotection (Chen et al., 1990). Using the Enzygnost (Siemens) kit, the EIA was also evaluated against the PRN at CDC (Bellini & Helfand, 2003). With the 120mIU/ml PRN cutoff for seroprotection, the EIA had 100% sensitivity and 91.0% specificity. And in a separate study of the Virion/Serion and IBL kits, the EIA demonstrated a relatively strong, statistically significant correlations of 0.878 ( $p<0.01$ ) and 0.850 ( $p<0.01$ ), respectively, with PRN (Mao, Zhu, & Jiang, 2009).

### 3 METHODS AND PROCEDURES

#### 3.1 Sample Preparation

Human serum samples from Tajikistan, the United States (US), and Bangladesh were used in this project, and data for each sample was acquired for the PRN, EIA, and MBA platforms. Samples from the US were residual diagnostic samples that were de-identified and approved for use by the National Center for Immunization and Respiratory Diseases (NCIRD) human research protections. Samples from Tajikistan were part of a 2010 serosurveillance study (Khetsuriani et al., 2013) of 1 to 24 year olds vaccinated at 9 or 12 months for first dose,



and 4 weeks later for second dose, according to WHO immunization guidelines for children (WHO, 2018). The Tajikistan study protocol was reviewed by the Human Subjects research coordinator at NCIRD and determined to be “program evaluation” and exempt from institutional review board approval. The protocol was also reviewed by the Ministry of Health in Tajikistan. Bangladesh samples were from a vaccine study on the administration of rotavirus vaccine with MMR (Zaman et al., 2016). Samples were acquired from patients before and after vaccination, in children between 9 months and 4 years; the first dose is administered at 9 months, and the second at 15 months (CDC, 2017). Approval was given by the Western Institutional Review Board and the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B), and post-vaccination samples are utilized for this assay comparison study. The MBA data for Bangladesh was run on the Luminex machine by Alexandria Mitchell, who prepared and ran the samples according to standard operating procedure as follows.

All sera were diluted into serum dilution buffer (3% bovine serum albumin [SIGMA], and 0.1% Tween 20 in phosphate buffered saline (PBS)) the day of or the day prior to running the MBA. The international rubella standard serum RUBI-1-94 (National Institute for Biological Standards and Control, NIBSC) was diluted in a 10 step 3-fold serial dilution starting at 1/400 for the standard curve, and control serum were diluted in a single point dilution of 1/800. The RUBI-1-94 standard was calibrated against the international standard serum for measles (WHO, NIBSC), and a standard curve is generated for measles. Tajikistan and U.S. samples were diluted at both 1/200 and 1/4000, and Bangladesh samples were diluted at 1/800. Non-magnetic microsphere beads covalently bound with measles antigen were used, with 4000 beads per well. The measles virus was purified using sucrose density gradient ultracentrifugation and

inactivated before being bound to the beads. Beads and serum were combined in a MultiScreen-BV filter Plate (Millipore®) and incubated at room temperature in the dark with shaking at approximately 800 revolutions per minute (rpm) for 45 minutes. A plate vacuum was used to remove liquid from the wells, while allowing the beads to remain. PBS was added to each well, and the beads were incubated at 1000rpm for one minute. The PBS was removed using a plate vacuum, and this wash step was repeated twice more. After washing, diluted secondary antibody (R-phycoerythrin conjugated Goat anti-human IgG, Jackson ImmunoResearch) is added to each well, and the plate is again wrapped in aluminum foil and incubated at 800rpm at room temperature. During this 30 minute incubation the fluorescently-tagged secondary antibody binds to any measles IgG antibodies in the samples. After incubation, the plate is washed three times as previously described. In the final step of sample and plate preparation, wash buffer is added to each well, and incubated at room temperature in the dark for five minutes at 1000rpm.

When the final incubation is complete, the plate is run using the Luminex machine. All samples were run on the Bio-Plex 100 or the Bio Plex 3D, which are both from the manufacturer Bio Rad (Hercules, CA). In the machine, the green “reporter” laser identifies secondary detection antibody, and the red “classify” laser distinguishes beads using internal dye of the beads. The measles antigen is covalently bound to microspheres, and as the beads are registered by the machine, the red laser detects the beads and the green laser reports secondary antibody. Beads that fall within the correct region are used to measure the fluorescence of the secondary antibody. The output pertinent to this project is the observed concentration of measles antibodies, which is determined when the standard curve converts

the fluorescence units of the secondary antibody into milli-international units per milliliter (mIU/ml). Results of the PRN are also based on a conversion against a standard, and values are reported in mIU/ml as well. Samples from the PRN and EIA were assessed using published methods (Albrecht, Herrmann, & Burns, 1981; ZEUS Scientific, 2017). All samples were tested by PRN by Sun Bae Sowers on the measles serology team, in the National Measles and Rubella Reference Laboratory at CDC. Tajikistan EIA data were assessed by the Siemens kit in Tajikistan; this kit is calibrated with the WHO standard, and results are converted and expressed in mIU/ml. EIA data for the United States and Bangladesh panels, run by Nobia Williams and Lijuan Hao, respectively, who are also on the serology team at CDC, were output using the Wampole kit (ZEUS Scientific, Inc.), which reports Immune Status Ratio (ISR) for each sample. ISR values are calculated by comparing the optical density (OD) of each EIA sample at a wavelength of 450 nanometers with a cutoff value calculated from the cutoff control in the kit. This kit is not calibrated to the WHO standard which would allow for conversion of these ISR values into mIU/ml.

### **3.2 Statistical Methods**

For the statistical analysis, data were used from the gold standard PRN, MBA, and EIA. The accuracy of the two comparing assays were evaluated using sensitivity, that corresponds to true positives and specificity, that corresponds to true negatives. Using logistic regression, the accuracy of probability of successes and failures was estimated by separate models comparing MBA to PRN and EIA to PRN. Country of origin was recorded for each sample, using data from Tajikistan (n=100), the United States (n=40), and Bangladesh (n=160). Age information was also

recorded for each sample and both country and age were evaluated as covariates. A total of 300 samples were used for this analysis, and two samples from the United States panel had missing MBA data. PRN and MBA observed concentration results for all countries are continuous. All EIA data are continuous as well, and ISR values from the Wampole kit range from 0-5+, with negative values (0-1.0 ISR), positive values (>1.1 ISR). Because of the large range in measles antibody concentration for each assay, in addition to having different units for the EIA data, all assay results were dichotomized using respective assay cutoff values. Results were either positive (greater than; seroprotected) or negative (less than; non-seroprotected) by PRN (cutoff= 120.00mIU/ml) and MBA (cutoff= 136.50mIU/ml). The cutoff value for the MBA was calculated using ROC curve analysis with results determined by the gold standard PRN by Dr. Melissa Coughlin, using GraphPad Prism 6 software. Tajikistan EIA data were dichotomized as positive or negative using a cutoff of 120.00mIU/ml, as given by the Siemen's kit. EIA results for the United States and Bangladesh samples were distinguished as positive (equal to or greater than) and negative (less than) using the cutoff of 1.1 ISR. Positive sample results for all assays were designated with a "1" and negative sample results with a "0".

Age is a continuous variable and was categorized into three groups: 0 to 5 years, 6 to 12 years, and 13 years and older. This is because individual sample ages were not given for Bangladesh samples, but all subjects range between infancy and 4 years of age. A boxplot was used to examine the distribution of age data for each country, and a scatterplot of PRN and MBA continuous data was created to show the wide range of possible values for both assays. Frequency tables were generated to compare dichotomous data from the MBA and EIA platforms with PRN results. Univariate procedures overall, by country, and by age group were

used for determining descriptive statistics as well as calculating positive predictive values (PPV) and negative predictive values (NPV). Additionally, McNemar's Test of Agreement was used to test for agreement between MBA and PRN, EIA and PRN, and MBA and EIA. Statistical modeling with logistic regression was used to estimate sensitivity and specificity for the MBA and EIA platform with PRN as the gold standard. Logistic regression models were fit for outcomes of both MBA and EIA to assess predicted probabilities with PRN, and for country as a fixed effect for MBA and EIA. Additional statistical models were fit with age included as a covariate for MBA and EIA. Results were considered statistically significant when  $p\text{-value} < 0.05$ , and all statistical analyses were conducted using SAS, version 9.3.

#### 4 RESULTS

Age had Mean= 8.79 years, SD= 10.21, and ranged from 0.25 years (three months) to 65 years across all countries. All samples from Bangladesh ranged from 0-4 years old, although individual sample ages were not known. Tajikistan sample ages ranged from 1-24 years (Mean= 9.62 years, SD= 7.04), and the United States sample ages ranged from 0.25-65 years (Mean= 25.87 years, SD= 16.65). Samples were distributed unequally across the three age categories: 0-5 years (n=208), 6-12 years (n=23), 13 years and older (n=68). The boxplots of the distribution of age by country (Figure A) also reflects this inequality in the range of ages for each country. The US has the largest range of ages, followed by Tajikistan and Bangladesh. Figure B depicts continuous MBA and PRN data with lines for respective cutoff values, and outlying values as high as 400,000mIU/ml. Most of the measles sample concentrations fall below 100,000mIU/ml for MBA and 50,000mIU/ml for PRN. When axis limits are in place (Figure C), a majority of the

samples are contained within the bottom left quadrant, indicating a “negative” result by both assays (n=33), and in the upper right quadrant, indicating a mutually “positive” result (n=215). A considerable portion of samples (n=44) lay in the bottom right quadrant, signifying a “negative” result by PRN, but “positive” result by MBA (false positives), and a few samples were falsely negative by MBA in the upper left quadrant (n=6).

All sample observed concentrations were extremely right-skewed, and descriptive statistics of median and inter-quartile range values were used to compare country data in Table 1. For Tajikistan, the median antibody concentration for PRN and MBA are 203.72mIU/ml and 283.67mIU/ml, respectively; the Tajikistan median for EIA was higher than PRN, at 435.82mIU/ml. For Bangladesh, the MBA and PRN median results are 1247.23mIU/ml compared with 232.50mIU/ml for PRN. Table 2 shows these descriptive statistics when country and age group are evaluated. MBA and PRN median values for Tajikistan samples aged 13 years and older are 399.28mIU/ml and 378.95mIU/ml, respectively. Across the three age groups and countries, EIA are 582.55mIU/ml, 399.28mIU/ml, and 378.95mIU/ml, respectively, for Tajikistan participants aged 13 and older. Following dichotomization, PRN had 74.33% (n=223) positive and 25.67% (n=77) negative samples, MBA had 86.91% (n=259) positive and 13.09% (n=39) negative samples, and EIA had 88.00% (n=264) positive and 12.00% (n=36) negative samples. McNemar’s Test of Agreement comparing MBA and PRN suggests disagreement between assays ( $p<0.0001$ ). This is also shown with EIA and PRN ( $p<0.0001$ ). When MBA and EIA were compared with McNemar’s Test of Agreement, the result was not significantly different ( $p=0.4669$ ).

The results of the logistic regression model with assay (Table 3) show the MBA and EIA have similar overall sensitivities with 97.29% (94.09, 98.76) and 97.76% (94.73, 99.06) when each compared to the gold standard PRN ( $p=0.7114$ ). Specificities of MBA and EIA were comparable as well ( $p=0.5157$ ). Positive predictive value is similar for the MBA at 83.01% compared to 82.58% ( $p=0.8887$ ) for EIA. When country effect is added (Table 4), these similarities are largely maintained. EIA sensitivity is higher for Tajikistan than MBA, with 95.89% compared to 93.68% ( $p=0.4839$ ). However, specificity for the MBA is better for this country, with 73.02% compared to 65.56% ( $p=0.2543$ ). For the United States, negative predictive value for the MBA is also higher than the EIA, at 77.78% compared to 70.00% ( $p=0.4354$ ), and positive predictive values for Bangladesh are very close, with 79.87% for EIA and 79.25% ( $p=0.9045$ ) for MBA. MBA sensitivity for Tajikistan and the United States is close as well ( $p=0.9761$ ), but when MBA sensitivities for Tajikistan and the United States are each compared with that of Bangladesh, they are significantly different at  $p=0.0016$  and  $p=0.0022$ , respectively. In Table 5, the United States' sensitivity for the MBA is higher for 0-5 and 13+ year age groups at 87.22% and 95.64%, respectively, compared with 84.37% ( $p=0.8650$ ) and 94.29% ( $p=0.8103$ ) for EIA. There were no statistically significant differences between the sensitivities of MBA and EIA for the first age category (0-5 years) for Tajikistan ( $p=0.7566$ ), the United States ( $p=0.8650$ ), and Bangladesh ( $p=1$ ). Between countries, MBA accuracy was not significantly different for Tajikistan and the United States ( $p=0.7642$ ), but was significantly different when Bangladesh was compared with these two countries ( $p=0.0001$  and  $p<0.0001$ , for Tajikistan and the US, respectively). For Tajikistan, positive predictive values and negative predictive values were the same for both assays in the 6-12 year age group, at 93.33% and 87.50%, respectively.

MBA specificity in the model without age and country versus in the model with country (Tajikistan) were significantly different ( $p < 0.0001$ ), although sensitivity was not ( $p = 0.0942$ ). MBA sensitivity and specificity in the country and age model (Tajikistan, 0-5 years) and the model with country only (Tajikistan) were not significantly different ( $p = 0.5222$  and  $p = 0.5823$ , respectively). However, when the country and age model (Tajikistan, 0-5 years) and the model without age and country were compared, MBA sensitivity and specificity were both significantly different ( $p = 0.0316$  and  $p < 0.0001$ , respectively).

## 5 DISCUSSION AND CONCLUSIONS

### 5.1 Discussion of Research Questions

In this study, the aims were to compare both the MBA and EIA with the gold standard PRN. Given the observed concentration of measles antibodies from samples run on each assay, these results were dichotomized according to each assay's respective cutoff value. Separate logistic regression models were used to evaluate the accuracy of MBA and EIA, when compared to the PRN. Source country for the samples was included in a model to understand its effect on this comparison, and age was added to the model to determine whether assay accuracy changed from one age group to another.

### 5.2 Study Strengths and Limitations

Study strengths included having multiple countries, and that samples from these countries consisted of slightly varying ages. Another strength was that this study is a pilot or feasibility study, as nothing in the literature would suggest a comparison of this nature has been previously undertaken. As such, the study was an attempt to understand whether a



comparison of this type could give insight into assay performance, and its accuracy in relation to results from a gold standard. The study is therefore not without limitations, which include not having individual age data for the Bangladesh samples, EIA values from two different kits resulting in different units for reporting concentrations, and the lack of equality in the number of positive and negative samples after dichotomization by cutoff values for Bangladesh.

### **5.3 Implications of Findings**

The MBA sensitivity is very high with low specificity when the data are not distinguished by country or age, and specificity percentages appear to improve as more information is added to the model, in the form of country and age information. By including additional sample information, MBA specificity was significantly different compared to that from models without country or age as fixed effects. However, because post-vaccination samples were used from Bangladesh, there was a lack of non-seroprotected samples to analyze; this makes specificity difficult to estimate. When compared with the PRN, the MBA performs similarly overall to the EIA with regards to sensitivity and specificity and in several groupings of country and age, these percentages are higher for the MBA. However, missing values, under-representation of age groups, and a small sample of source countries makes this difficult to infer. The positive and negative predictive values for MBA were relatively high and consistent for all models fit, and this is the same for EIA as well. Many of the benefits of the MBA over conventional assays (that it is faster, requires much less sample volume, and can multiplex several antigens simultaneously), have led to its increased application in research and this should be considered. This study demonstrates the comparability of the MBA with the EIA, through comparability of the sensitivity, specificity, and positive and negative predictive values for these assays.

Although the EIA is currently the most commonly used assay for determining measles antibody concentration, results from this study suggest the MBA is a suitable alternative.

#### **5.4 Recommendations and Future Strategies**

Recommendations and future strategies are to compare both the MBA and EIA to PRN with a larger overall and country-specific sample size, an increased number of countries, and with more demographic sample information if possible. Possible benefits and disadvantages of implementing the MBA in place of the EIA or PRN should be evaluated, and this includes the cost of the MBA, how to improve MBA specificity, whether MBA use is situation specific, what other antigens if any can be multiplexed with the MBA, and how to incorporate the MBA into research settings unfamiliar with the technology. Logistic regression models can then be fit to compare the assays and assess sensitivity and specificity. This will allow for more representative samples and stronger conclusions to be inferred.

#### **5.5 Conclusions**

When compared to PRN, MBA sensitivity was high, and by including Bangladesh, specificity appeared to improve when additional sample information of source country and age were added to the model. Country and age did not have significant effects on MBA and EIA accuracy for Tajikistan and the US. However, the lack of non-seroprotected Bangladesh samples in the analysis may be influencing the significant difference in MBA specificity across models with and without fixed effects, and the significant difference of MBA sensitivity between countries. For datasets containing ample seroprotected and non-seroprotected samples, including country and age information in the models may affect MBA specificity differently. Moreover, because the US, Tajikistan, and Bangladesh have different vaccination

recommendations, this could also affect how age data are interpreted. For serosurveillance, age data is important to include because low measles concentrations by MBA could be an indication that certain age groups were missed during vaccination. In conclusion, the MBA performed similarly with EIA, and these results suggest the MBA can be used in settings where the EIA is currently used. This was a pilot study, and if weaknesses and implications are addressed, future studies can re-evaluate this comparison of assays and include more information to make more informed inferences.

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## APPENDICES

### Appendix A

#### *MPH Core Competencies*

Upon completion of the MPH degree, all students will have a mastery of appropriate theory, knowledge and skills in applied public health and public health research as evidenced by the graduate's ability to:

1. Describe the roles biostatistics serves in the discipline of public health and the function of ethics in biostatistics practice.
2. Describe basic concepts of probability, random variation and commonly used statistical probability distributions.
3. Apply basic (univariate and bivariate) descriptive and inferential techniques commonly used with public health data.
4. Critically evaluate the application, presentation, and interpretation of statistical analyses in the public health studies.
5. Describe major environmental and occupational contaminants including biological, chemical and physical agents and discuss effects of exposure to these contaminants on human health.
6. Identify important susceptible human sub-populations with respect to environmental exposures and the sources of variability.
7. Analyze approaches to assessing, preventing and controlling environmental hazards that pose risks to human health.
8. Calculate and interpret common epidemiologic measures to draw appropriate inferences.
9. Critically evaluate strengths and weaknesses of epidemiologic methods.
10. Communicate epidemiologic concepts in both technical and lay language by explaining trends and patterns of health-related events and the importance of epidemiology in health policy, disease prevention, and health promotion.
11. Identify and critically discuss the organization and financing of the health services and public health systems in the United States, with emphasis on the consequences for vulnerable populations.
12. Apply evidence-based principles to critically evaluate current policies and practices in healthcare delivery and in public health systems including present and future healthcare reform proposals to address the quality, accessibility and cost of our health systems.
13. Describe how social and behavioral risk factors contribute to individual and public health outcomes.
14. Develop and evaluate social and behavior interventions, especially through community participatory research in diverse communities.
15. Apply evidence-based approaches in the development and evaluation of social and behavioral science interventions.

*MPH Biostatistics Concentration Competencies*

Students in the Master of Public Health program with a concentration in Biostatistics will be expected to demonstrate competence in the following areas:

BSTP 1. Apply advanced (multivariate) descriptive and inferential techniques used with public health data.

BSTP 2. Describe preferred methodological alternatives to commonly used statistical methods when assumptions are not met.

BSTP 3. Distinguish among the different measurement scales and the implications for selection of statistical methods to be used based on these distinctions.

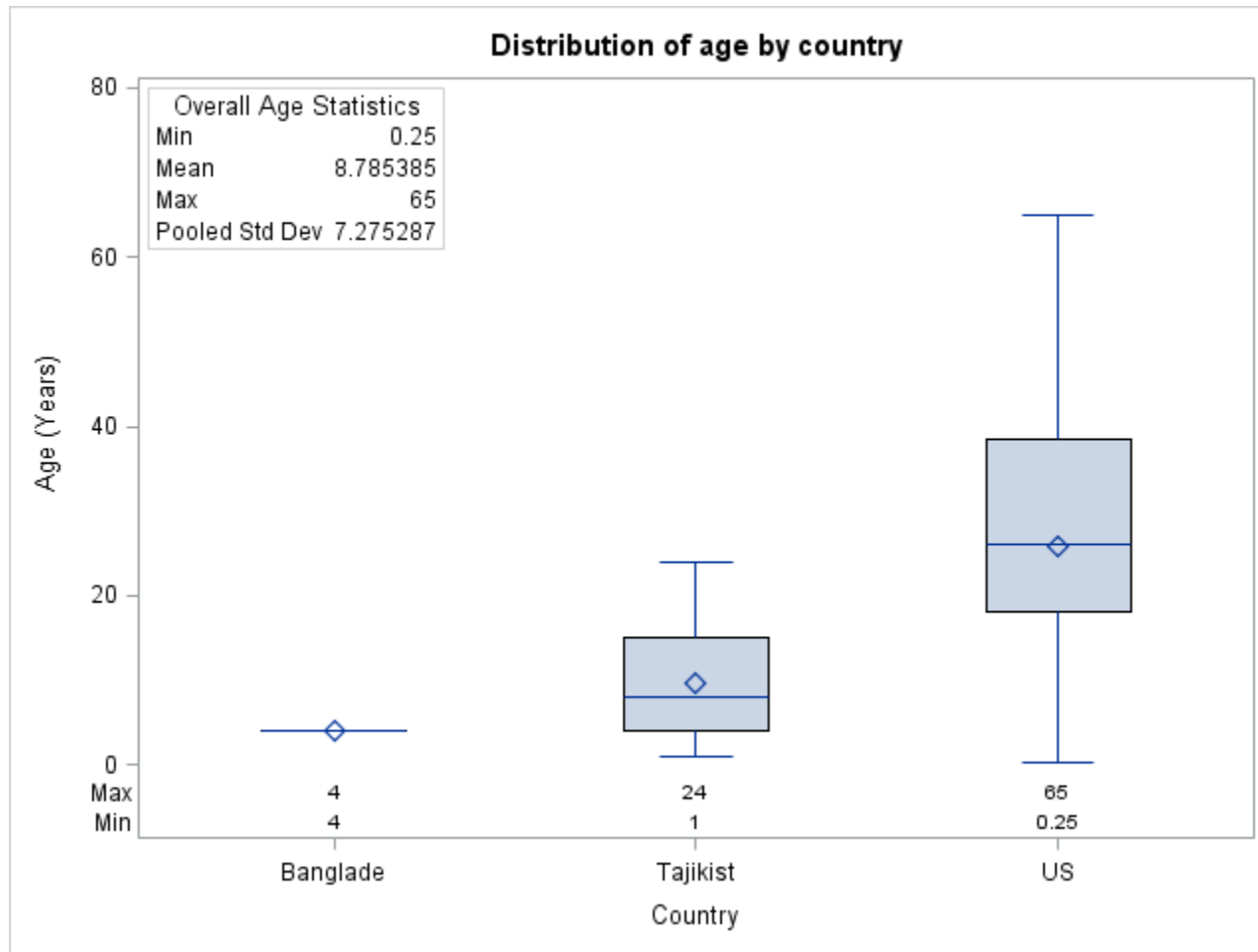
BSTP 4. Apply basic informatics techniques (storage, access, management, organization, visualization, and evaluation of public health data) in public health research.

BSTP 5. Describe different public health study designs, measures, and the appropriate statistical analyses for answering particular research questions.

BSTP 6. Interpret results of statistical analyses found in public health studies.

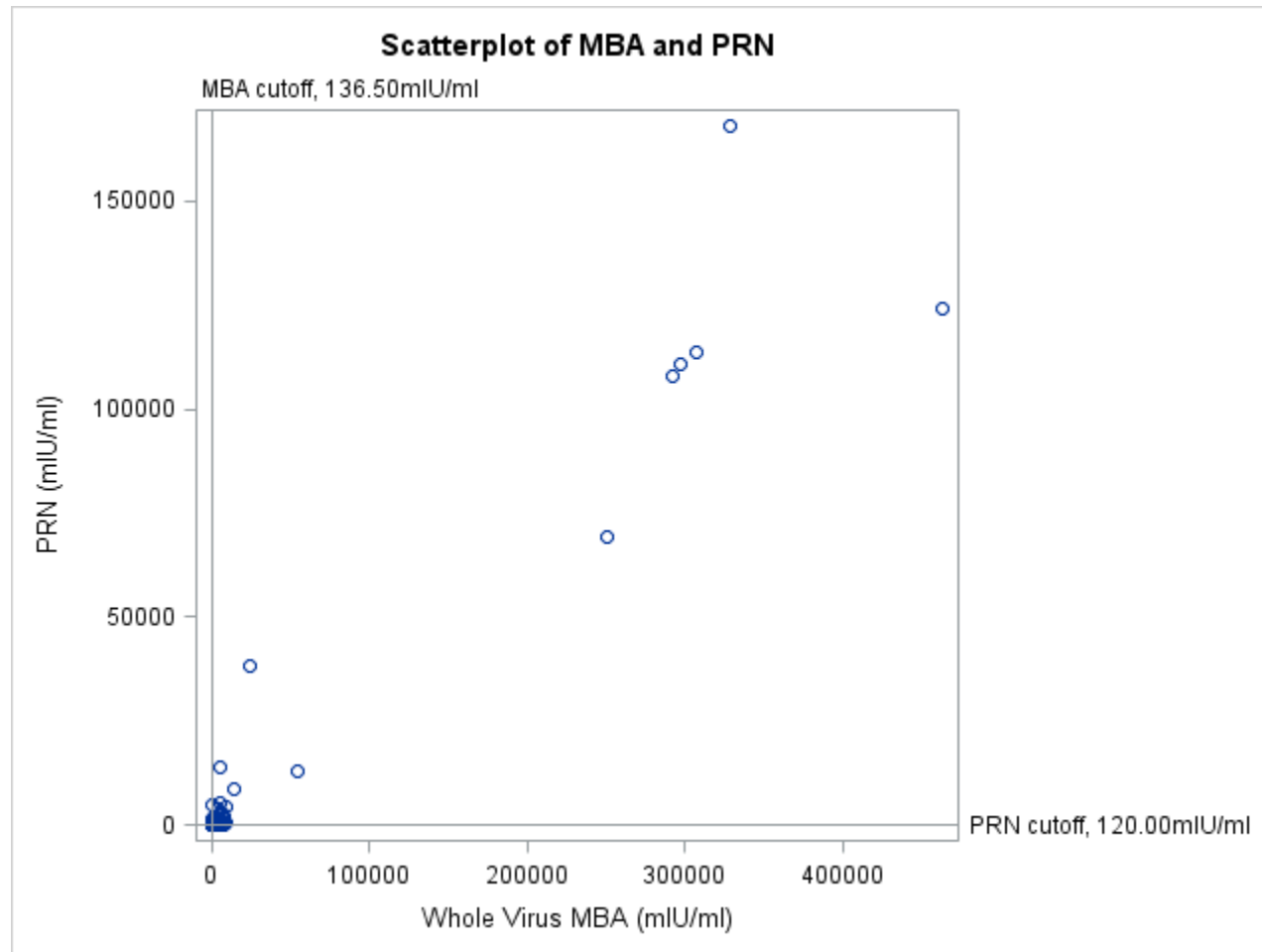
BSTP 7. Develop written and oral presentations based on statistical analyses for both public health professionals and educated lay audiences.

**Figure A. Distribution of Age by Country (Bangladesh n=160, Tajikistan n=100, US n=40)**

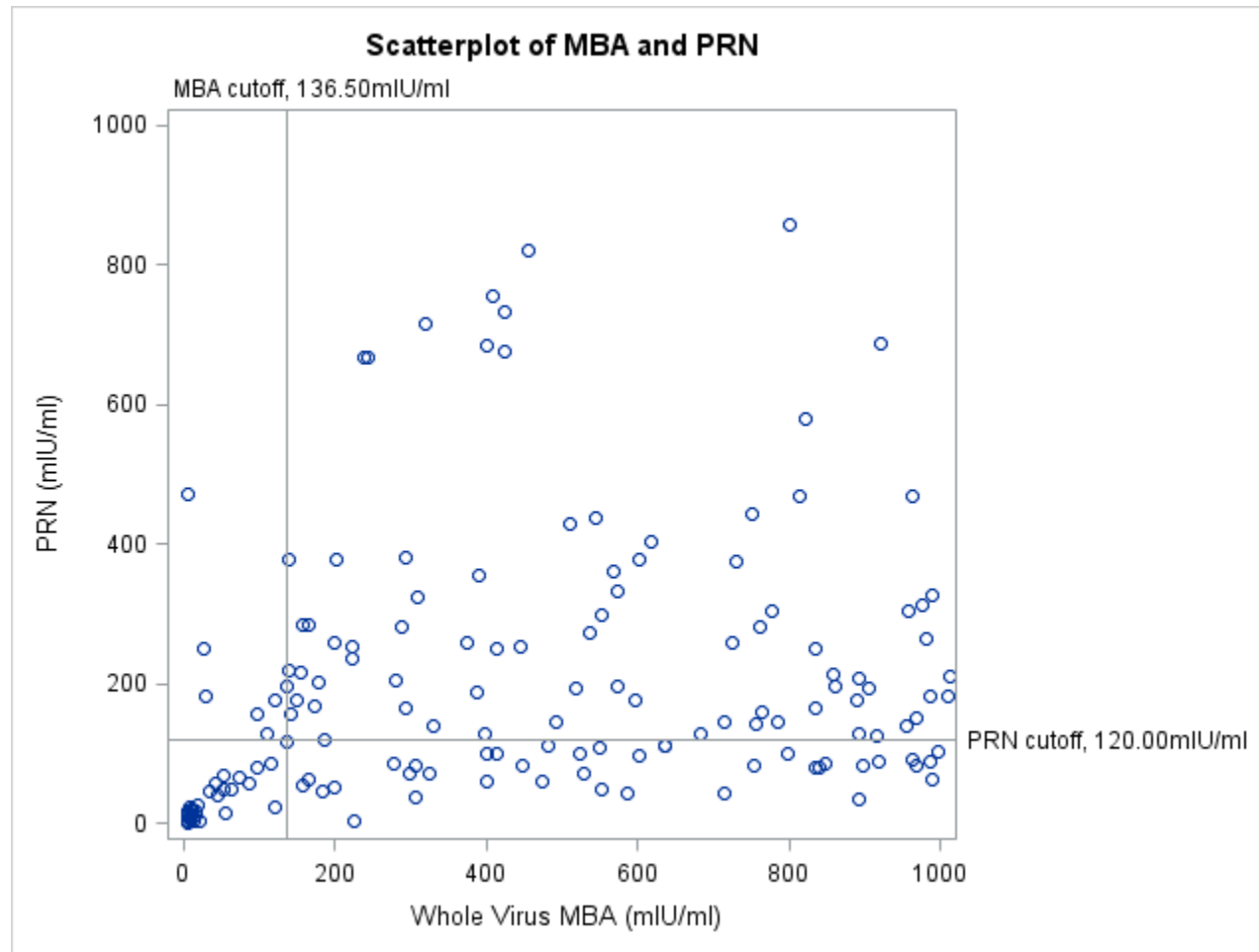




**Figure B. Scatterplot of MBA and PRN**



**Figure C. Scatterplot of MBA and PRN with Axis Restrictions**



**Table 1. Descriptive Statistics of Country by Assay**

Measles Antibody Concentration				
	Country	N	Median	Interquartile Range (25 <sup>th</sup> , 75 <sup>th</sup> )
PRN	Tajikistan (mIU/ml)	100	203.72	(62.26, 551.35)
	United States (mIU/ml)	40	1161.00	(133.50, 13580.50)
	Bangladesh (mIU/ml)	160	232.50	(131.00, 396.50)
MBA	Tajikistan (mIU/ml)	100	283.67	(112.78, 764.50)
	United States (mIU/ml)	38	788.75	(166.02, 9479.88)
	Bangladesh (mIU/ml)	160	1247.23	(890.16, 2022.05)
EIA	Tajikistan (mIU/ml)	100	435.82	(129.02, 1781.60)
	United States (ISR)	40	2.22	(1.17, 4.91)
	Bangladesh (ISR)	160	1.93	(1.61, 2.19)

**Table 2. Descriptive Statistics of Country and Age by Assay**

Measles Antibody Concentration					
	Country	Age (Years)	N	Median	IQR (25 <sup>th</sup> , 75 <sup>th</sup> )
PRN	Tajikistan (mIU/ml)	0-5	39	175.20	(26.64, 285.49)
		6-12	23	181.09	(44.90, 444.18)
		13+	37	378.95	(138.68, 687.63)
	United States (mIU/ml)	0-5	9	8.00	(2.00, 16.00)
		6-12	0		
		13+	31	1797.00	(375.00, 69225.00)
	Bangladesh (mIU/ml)	0-5	160	232.50	(131.00, 396.50)
		6-12	0		
		13+	0		
MBA	Tajikistan (mIU/ml)	0-5	39	157.60	(19.30, 537.71)
		6-12	23	225.00	(55.02, 1009.48)
		13+	37	399.28	(198.17, 1129.88)
	United States (mIU/ml)	0-5	9	8.98	(6.29, 22.24)
		6-12	0		
		13+	29	1320.63	(349.49, 24082.25)
	Bangladesh (mIU/ml)	0-5	160	1247.23	(890.16, 2022.05)
		6-12	0		
		13+	0		
EIA	Tajikistan (mIU/ml)	0-5	39	434.58	(133.72, 1628.43)
		6-12	23	435.82	(129.02, 1781.60)
		13+	37	582.55	(249.73, 2157.48)
	United States (ISR)	0-5	9	1.03	(0.22, 1.73)
		6-12	0		
		13+	31	2.74	(1.58, 8.70)
	Bangladesh (ISR)	0-5	160	1.93	(1.61, 2.19)
		6-12	0		
		13+	0		

**Table 3. Sensitivity and Specificity of MBA compared to PRN and EIA compared to PRN**

Comparison with PRN (Gold Standard)								
		Negative	Positive	Total	Sensitivity (%) 95% CI	Specificity (%) 95% CI	PPV (%)	NPV (%)
MBA vs. PRN	Negative	33	6	39				
	Positive	44	215	259				
	Total	77	221	298	97.29 (94.09, 98.76)	42.86 (45.92, 67.68)	83.01	84.62
EIA vs. PRN	Negative	31	5	36				
	Positive	46	218	264				
	Total	77	223	300	97.76 (94.73, 99.06)	40.26 (48.48, 70.06)	82.58	86.11

**Table 4. Sensitivity and Specificity of MBA compared to PRN and EIA compared to PRN, with Country Effect**

MBA vs. PRN					EIA vs. PRN				
Country	Negative	Positive	Total		Negative	Positive	Total		
Tajikistan				Sensitivity (%)				Sensitivity (%)	
				(95% CI)				(95% CI)	
Negative	<b>26</b>	<b>3</b>	29	Specificity (%)	<b>23</b>	<b>2</b>	25	Specificity (%)	
				(95% CI)				(95% CI)	
Positive	<b>8</b>	<b>63</b>	71	PPV (%)	<b>11</b>	<b>64</b>	75	PPV (%)	
Total	34	66	100	NPV (%)	34	66	100	NPV (%)	
United States*				Sensitivity (%)				Sensitivity (%)	
				(95% CI)				(95% CI)	
Negative	<b>7</b>	<b>2</b>	9	Specificity (%)	<b>7</b>	<b>3</b>	10	Specificity (%)	
				(95% CI)				(95% CI)	
Positive	<b>3</b>	<b>26</b>	29	PPV (%)	<b>3</b>	<b>27</b>	30	PPV (%)	
Total	10	28	38	NPV (%)	10	30	40	NPV (%)	
Bangladesh**				Sensitivity (%)				Sensitivity (%)	
				(95% CI)				(95% CI)	
Negative	<b>0</b>	<b>1</b>	1	Specificity (%)	<b>1</b>	<b>0</b>	1	Specificity (%)	
				(95% CI)				(95% CI)	
Positive	<b>33</b>	<b>126</b>	159	PPV (%)	<b>32</b>	<b>127</b>	159	PPV (%)	
Total	33	127	160	NPV (%)	33	127	160	NPV (%)	

\*MBA data missing for two US subjects \*\*Non-seroprotected samples were not analyzed for Bangladesh

**Table 5. Sensitivity and Specificity of MBA compared to PRN and EIA compared to PRN, with Country and Age Effects**

		MBA vs. PRN					EIA vs. PRN				
Country	Age (Years)	N	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)	N	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)
Tajikistan*	0-5	39	90.56 (75.81, 96.71)	77.55 (58.36, 89.49)	83.33	93.33	39	92.51 (79.20, 97.57)	73.69 (55.07, 86.49)	80.77	100.00
	6-12	23	89.28 (69.54, 96.82)	79.90 (51.52, 93.70)	93.33	87.50	23	95.58 (88.50, 98.38)	61.57 (44.06, 76.52)	93.33	87.50
	13+	37	96.85 (89.94, 99.07)	51.83 (25.81, 76.90)	90.32	83.33	37	97.42 (91.69, 99.23)	47.82 (24.33, 72.31)	84.85	75.00
United States**	0-5	9	87.22 (60.33, 96.84)	82.91 (54.23, 95.21)	100.00	100.00	9	84.37 (54.82, 96.00)	86.51 (59.59, 96.54)	100.00	100.00
	6-12	0					0				
	13+	29	95.64 (84.88, 98.84)	0.00 (NA)	88.89	0.00 (NA)	31	94.29 (82.41, 98.31)	0.00 (NA)	89.29	0.00 (NA)
Bangladesh***	0-5	160	99.92 (99.24, 99.99)	0.00 (NA)	79.25	0.00 (NA)	160	99.92 (99.25, 99.99)	2.72 (0.38, 16.95)	79.87	100.00
	6-12	0					0				
	13+	0					0				

\*Age data missing for one Tajikistan subject \*\*US missing 2 MBA subject results for Age 13+ category \*\*\*Non-seroprotected samples were not analyzed for Bangladesh